

Table 1

Percent weight of lutein and  $\beta$ -carotene in the snails maintained on the yolk-lettuce (Y-L) diet or lettuce (L) diet.

Pigment	Days after the cultures were started	Y-L*	L*
Lutein	14	0.0213 $\pm$ 0.0037 <sup>†</sup>	0.0624 $\pm$ 0.015
	20	0.00833 $\pm$ 0.0039 <sup>a,†</sup>	0.0198 $\pm$ 0.0045 <sup>b,‡</sup>
$\beta$ -carotene	14	0.0410 $\pm$ 0.015 <sup>†</sup>	0.0600 $\pm$ 0.026
	20	0.00840 $\pm$ 0.0030 <sup>c, **</sup>	0.0530 $\pm$ 0.0064 <sup>†</sup>

\* Snail bodies: mean (weight %)  $\pm$  standard error; n = 5 individual snails for each sample (except where indicated).\*\* Concentration significantly reduced (Student's t-test,  $P < 0.05$ ) compared with snails on the L diet.<sup>a</sup> Two data points were below the limit of quantification. Weight % values of 0.00340 and 0.00575 were used for statistical analysis.<sup>b</sup> One data point was below the limit of quantification. Weight % value of 0.00832 was used for statistical analysis.<sup>c</sup> Three data points were below the limit of quantification. Weight % values of 0.00288, 0.00311, and 0.00510 were used for statistical analysis.<sup>†</sup> n = 3.<sup>‡</sup> n = 4.

air for about 30 sec. The plates were developed to a distance of about 7 cm past the concentrating zone-bonded silica gel interface with petroleum ether-acetonitrile-methanol (10 + 20 + 20 v/v) in a rectangular Camag (Wilmington, North Carolina) TLC twin-trough chamber. The chamber was covered with aluminum foil, lined with a saturation pad (Analtech, Newark, Delaware), and equilibrated with the mobile phase for at least 15 min before inserting the plate. Approximately 40 mL of mobile phase was required for each development. The required development time was about 20 min. The plates were briefly dried in air for about 2 min after development. The pigments were detected in visible light as colored bands on a white background.

Quantitative densitometric analysis was performed with a Camag TLC Scanner II with the tungsten light source (set at 448 nm for lutein and 455 nm for  $\beta$ -carotene), slit width 4, slit length 4, and scanning rate 4 mm s<sup>-1</sup>. The CATS-3 software was used to generate a linear regression calibration curve relating the weights of the standard zones (0.0400–0.160  $\mu$ g) to their peak areas. The analyte weight in the sample aliquot with a scan area closest to that of the average of the middle two standard zones was determined by automatic interpolation from the calibration curve, on the basis of its peak area. The weight percents of pigments in the snail whole-body were calculated using the equation described earlier (Sherma et al., 1992).

For quantification of some samples, dilution or concentration were required to obtain scan areas that would be bracketed within the calibration curve. An appropriate correction factor was then included in the calculation. On six occasions after the maximum possible degree of concentration, the largest spotted sample yielded a zone whose area was less than the scan area of the lowest standard zone. Therefore, the exact quantities of the pigment in these zones could not be determined because they

were below the experimental quantification limit, which ranged from 0.00575–0.0166 weight percent of the pigments for the conditions under which the analyses were performed. The reconstitution volume was 200  $\mu$ L for all six samples, with 4.00, 8.00, 4.00, 8.00, 8.00, and 8.00  $\mu$ L aliquot spotted, respectively. For these zones, a concentration of one-half of the limit of quantification was included in the data for statistical calculations (Cline et al., 1999) (see Table 1).

## RESULTS AND DISCUSSION

By comparison with the migration of standards, lutein and  $\beta$ -carotene were identified in chromatograms of the whole snail body extracts from snails fed both the Y-L and L diets at  $R_f$  values of 0.45 and 0.070, respectively. The sample also contained several other pigment zones with different  $R_f$  values, one of which was qualitatively determined to be chlorophyll A.

Table 1 lists quantitative data for lutein and  $\beta$ -carotene in the snails fed both the Y-L and L diet for 14 and 20 days (n = 5 for each sample). The mean values of lutein in the snails on the L diet were  $\times 3$  and  $\times 2.5$  at 14 and 20 days, respectively, compared to snails on the Y-L diet. Likewise, the mean values of  $\beta$ -carotene in snails on the L diet were  $\times 1.5$  and  $\times 6.6$  at 14 and 20 days, respectively, compared to the snails on the Y-L diet. However, the only results that were significantly different (Student's t-test,  $P < 0.05$ ) were those for  $\beta$ -carotene values in the snail tissues 20 days after the cultures were started. By this time, snails on the Y-L diet had significantly reduced amounts of  $\beta$ -carotene than snails on the L diet. The weight percents of lutein (n = 1) from the hen's egg yolk and Romaine leaf lettuce were 0.0730 and 0.253, respectively. The weight percents of  $\beta$ -carotene (n = 1) from the yolk and lettuce were 0.0140 and 0.0830, respectively. The concentration of lutein in the lettuce was approx-

imately  $\times 3.5$  that in the egg yolk. The concentration of  $\beta$ -carotene in the lettuce was approximately  $\times 8$  that in the egg yolk.

The amount of lutein and  $\beta$ -carotene pigments in general reflected the relative amounts of these pigments in the diets. Thus, concentrations of both lutein and  $\beta$ -carotene were higher in the lettuce than the yolk diet, and these higher values were reflected in snails on the L versus Y-L diet. These results are in general accord with a previous study by Eidam et al. (2001) that compared various analytes in the tissue and hemolymph of *Biomphalaria glabrata* fed a diet of Romaine lettuce leaf versus the midrib of the Romaine lettuce. The leafy portion of the Romaine lettuce contained significantly greater amounts of neutral lipids, phospholipids, lipophilic pigments, and carbohydrates than did the midrib portion of the Romaine lettuce. Higher values in these analytes were seen in the tissues and hemolymph of the *B. glabrata* snails fed the leafy portion of the Romaine lettuce. The adage "you are what you eat" is applicable to the *B. glabrata* snails.

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## NOTES, INFORMATION & NEWS

### *Kalidos griffithshauchleri*, sp. nov., Madagascar's Largest Helicarionid Snail (Pulmonata)

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#### Introduction

Owen Griffiths of Mauritius (along with his associates and assistants) was a major participant in the author's 1992–1996 survey and inventory of Madagascar's land mollusks. Griffiths' unique and strongest contribution was in surveying the Reserve Naturelle Integrale de Tsingy de Bemaraha, a little-explored limestone karst region in west-central Madagascar. After some preliminary scouting in 1992 and 1993, Griffiths led expeditions in 1995 and 1996 into the southern and central-plus-northern parts of Bemaraha, respectively (Griffiths, 1995, 1996). Among the many new species of land snails resulting from Griffiths' Bemaraha collections (in Emberton, 1999a, b, 2001, 2002, in press) is the remarkable new *Kalidos* described herein.

The genus *Kalidos* Gude, 1911, is endemic to Madagascar; its sister group has been predicted from biogeographic considerations to lie among the ariophantines of India (Emberton & Rakotomalala, 1996). The *Faune de Madagascar* (Fischer-Piette et al., 1994) listed 71 *Kalidos* species (23 new), Emberton (1994) added one new species, and Emberton & Pearce (2000) added four new species. Thus this current new species brings the total to 77.

The author's 1992–1996 survey and inventory of Madagascar yielded over 2000 lots of *Kalidos* species. Only 438 of these lots have been identified so far, and the 1995–1996 Bemaraha *Kalidos* materials have not been reached yet in this process. However, three specimens of *K. griffithshauchleri*, sp. nov. that were collected in 1992–1993 were sent to the author's attention some time ago and merit description now—in advance of the author's plan to monograph the genus—because of this species' unique size and its conservation implications for Bemaraha Reserve.

The author's identifications of 438 of the some 2000 lots of *Kalidos* have yielded 65 presumed species, of which 42 seem new and undescribed (Emberton, unpublished). Thus Madagascar's total *Kalidos* species now in collections is likely to be at least 250 (contradicting Emberton & Rakotomalala's 1996: table II estimate of "75?"). Most of those species are small, and none begins to approach this new species in its gigantic shell size. All other known and collected Madagascan helicarionids, with the exception of this gigantic Bemaraha species, are much smaller in size

(Fischer-Piette et al., 1994; Emberton 1994; Emberton & Pearce, 2000; Emberton, unpublished).

#### Systematics

Higher classification follows Ponder & Lindberg (1997), Nordsieck (1986), and Vaught (1989). Type materials are placed in the Florida Museum of Natural History, University of Florida, Gainesville (UF) and the Australian Museum, Sydney (AMS). Description follows the format applied to other *Kalidos* by Emberton & Pearce (2000).

#### Class GASTROPODA

#### Clade HETEROBRANCHIA

#### Clade PULMONATA

#### Order STYLOMMATOPHORA

#### Suborder SIGMURETHRA

#### Infraorder HELICIDA

#### Superfamily HELICARIONOIDEA

#### Family HELICARIONIDAE

#### Subfamily ARIOPHANTINAE

#### Genus *Kalidos* Gude, 1911

#### *Kalidos griffithshauchleri* Emberton, sp. nov.

(Figure 1)

*Kalidos* sp. 1, Griffiths, 1995; Griffiths, 1996.

**Diagnosis:** Unique within the genus for its large initial whorls and very rapid whorl-expansion rate producing a gigantic adult shell. *Kalidos griffithshauchleri*, sp. nov. is most similar to *K. bathensis* (Robson, 1914), from which it differs in both its larger initial whorls (diameters of first and first-plus-second whorls = 2.2 mm and 5.1 mm versus 1.7 mm and 3.8 mm) and its looser coiling (whorls/ $\ln$ [diameter] 1.51–1.60 versus 1.76).

**Holotype:** UF285447 (1 adult), Owen Griffiths lot A1680: Madagascar: near Tsingy de Bemaraha: 15 km east of Antsalova: in cave mouth, April 1992.

**Paratypes:** UF285448 (1 adult), type lot. AMS C. 204776 (1 adult), Owen Griffiths lot A1737: Madagascar: near Tsingy de Bemaraha: southeast of Antsalova: near Tsiandro: in cave mouth, April 1993.

#### Description of holotype:

*Shell Size and Shape.* Shell rather thick and robust for